

# ***In vitro* inhibition of Hepatitis C virus protease and antioxidant by flavonoid glycosides from the Saudi costal plant *Sarcocornia fruticosa***

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## **ABSTRACT**

A new flavonol triglycoside, rhamnazin 3-O-2<sup>G</sup>-rhamnorutinoside or rhamnazin 3-O-(2'',6''-O- $\alpha$ -di-rhamnosyl)- $\beta$ -glucoside (**1**) was isolated along with known flavonols, rhamnazin 3-O-rutinoside (**2**), rhamnazin 3-O-(6''-O- $\alpha$ -rhamnosyl)- $\beta$ -galactoside (**3**), isorhamnetin 3-O-(6''-O- $\alpha$ -rhamnosyl)- $\beta$ -galactoside (**4**), isorhamnetin 3-O-(2'',6''-O- $\alpha$ -di-rhamnosyl)- $\beta$ -galactoside (**5**), and isorhamnetin (**6**), and allantoin (**7**) from the aqueous methanol extract of *Sarcocornia fruticosa* leaves. Spectral analyses (UV, MS, and NMR) and acid hydrolysis were used to determine the structures. These compounds in this study except **6** were reported for the first time from the genus *Sarcocornia*. The extract and flavonol glycosides (**1-5**) were evaluated for antioxidant and inhibition of HCV protease enzyme. Rhamnazin triglycoside (**1**) was shown to have a potent HCV protease inhibitor with IC<sub>50</sub> value 8.9  $\mu$ M, while isorhamnetin di- and triglycosides (**4** and **5**) were effectively scavenged DPPH radicals with IC<sub>50</sub> values 3.8 and 4.3  $\mu$ M, respectively.

**Keywords:** *Sarcocornia fruticosa*, flavonol glycosides, rhamnazin 3-O-2<sup>G</sup>-rhamnorutinoside, antioxidant, anti-HCV protease

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Table S1. NMR spectral data of isolated flavonol glycosides **1** and **5** in DMSO-*d*<sub>6</sub>

C/H	<b>5</b>			<b>1</b>			COSY	HMBC ( <sup>2</sup> J and <sup>3</sup> J)
	δ <sub>C</sub>	δ <sub>H</sub>	Hz	δ <sub>C</sub>	δ <sub>H</sub>	Hz		
2	155.5			157.3				
3	132.6			133.1				
4	176.9			177.7				
5	157.1			161.2				
6	100.4	5.98	br	98.4	6.33	br	H-8	C-10, C-8, C-7, C-5
7	161.5			165.5				
8	94.7	6.18	br	92.8	6.69	br	H-6	C-10, C-6, C-7, C-9
9	157.1			156.8				
10	104.3			105.4				
1'	121.6			121.2				
2'	114.1	7.97	br	113.6	7.85	br	-	C-6', C-2, C-4', C-3'
3'	147.3			147.3				
4'	149.8			150.1				
5'	115.5	6.85	d, 8.4	115.6	6.91	d, 8.4	H-6'	C-1', C-4', C-3'
6'	121.9	7.43	d, 8.4	122.7	7.53	d, 8.4	H-5'	C-2', C-2, C-4'
Gal/Gluc-1"	99.4	5.66	d, 7.8	99.3	5.59	d, 7.2	H-2"	C-3, C-2"
2"	75.6	3.76	m	78.0	3.45	m	H-1"	C-1", C-1"', C-3"
3"	74.1	3.59	m	77.3	3.42	-**		C-2"
4"	68.9	3.59	m	70.9	3.45	m		C-3"
5"	73.6	3.60	m	76.2	3.64	m	H-6b"	-
6a"	65.3	3.58	d, 12	67.3	3.66	d, 9.6		C-1"', C-1''( <sup>4</sup> J)
6b"	65.3	3.28*	d, 13	67.3	3.28	d, 9.0	H-5"	C-1"', C-5"
Rh-1'''	101.2	4.95	br	101.1	5.02	br		C-2'', C-2''', C-5'''
2'''	71.0	3.28	d, 10	70.7	3.34*	br		C-1'''
3'''	71.1	3.44	d, 9.0	71.0	3.12*	m	H-4'''	C-2'''
4'''	72.3	3.06*	dd, 9.6	72.1	3.03*	dd, 10.2	H-3'''	C-5'''
5'''	68.7	3.38*	d, 9.0	68.7	3.19	d, 7.8	H-6'''	C-4'''
6'''-CH <sub>3</sub>	18.3	1.03	d, 5.4	18.1	0.92	d, 6.0	H-5'''	C-5''', C-4'''
Rh-1''''	100.4	4.38	br	101.3	4.36	br	H-2''''	C-6'', C-2''', C-5'''
2''''	70.8	3.28	d, 10	70.7	3.34	-	H-1''''	C-1''''
3''''	71.0	3.38	d, 9.0	71.0	3.12	m		C-4''''
4''''	72.2	3.06	dd, 9.0	72.1	3.02	dd, 9.6	H-5''''	C-5''''
5''''	68.7	3.38	m	68.7	3.71	d, 7.8	H-4''''	C-4''''
6''''-CH <sub>3</sub>	17.5	0.67	d, 5.4	17.6	0.71	d, 6.0	H-5''''	C-5''', C-4''''
7-OCH <sub>3</sub>	-			56.5	3.82	s		C-7
3'-OCH <sub>3</sub>	56.2	3.84	s	56.1	3.82	s		C-3'
5-OH	-			-	12.59	s		C-10, C-5

\*Overlap sugar proton signals and \*\*H-signal under water-DMSO signal were detected by HSQC correlations

Table S2: NMR spectral data of isolated allantoin (**7**) in DMSO-*d*<sub>6</sub>

C/N No.	$\delta_C$	$\delta_H$	<i>J</i> Hz	HMBC	$\delta_N$ : <sup>15</sup> N-HSQC	<sup>15</sup> N-HMBC
2	157.2					
4	157.8					
6	174.0					
7	62.8	5.24, 1H,	dd, 1.2 & 8.16	C-6, C-4		
1-NH		7.02, 1H,	d, 8.16	C-2, C-6, C-7	91.3	
3-NH		10.51, 1H,	br		148.1	
5-NH		8.06, 1H,	s	C-2, C-6, C-7	101.4	3-NH
7-NH <sub>2</sub>		5.84, 2H,	s	C-7	77.9	1-NH

## Spectral data of flavonol glycosides (**2-5**)

### *Rhamazin 3-O-(6''-O- $\alpha$ -rhamnosyl)- $\beta$ -galactoside (2)*

Pale yellow powder; UV  $\lambda_{\max}$  (MeOH): 255, 357; (NaOMe): 264, 420; (AlCl<sub>3</sub>): 269, 301, 379, 399; (AlCl<sub>3</sub>/HCl): 269, 368, 403; (NaOAc): 258, 367, 419; (NaOAc/H<sub>3</sub>BO<sub>3</sub>): 256, 358. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>):  $\delta$  12.91 (1H, s, 5-OH), 7.77 (1H, br; H-2'), 7.63 (1H, d, *J* = 7.4 Hz; H-6'), 6.39 (1H, br; H-5'), 6.24 (1H, br; H-8), 5.74 (1H, br; H-6), 5.14 (1H, d, *J* = 5.1 Hz; H-1''), 4.35 (1H, br, H-1'''), 3.83 (3H, s, 7-OCH<sub>3</sub>), 3.70 (3H, s, 3'-OCH<sub>3</sub>), 1.00 (3H, d, *J* = 6 Hz; H-6'''); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>):  $\delta$  175.8 (C-4), 166.7 (C-2), 164.8 (C-7), 159.1 (C-5), 156.6 (C-9), 155.0 (C-4'), 149.8 (C-3'), 131.9 (C-3), 126.1 (C-6'), 122.4 (C-1'), 118.4 (C-5'), 113.3 (C-3'), 104.8 (C-10), 104.6 (C-1''), 101.8 (C-1'''), 97.8 (C-6), 92.9 (C-8), 76.7 (C-3''), 75.8 (C-5''), 74.8 (C-2''), 72.4 (C-4'''), 70.9 (C-3'''), 70.7 (C-2'''), 70.0 (C-4''), 68.8 (C-5'''), 67.6 (C-6''), 56.3 (7-OCH<sub>3</sub>), 55.3 (3'-OCH<sub>3</sub>), 18.2 (C-6'''). ESI-MS: *m/z* 661 [M+Na]<sup>+</sup> (100%).

### *Rhamazin 3-O-(6''-O- $\alpha$ -rhamnosyl)- $\beta$ -galactoside (3)*

Yellow powder; UV  $\lambda_{\max}$  (MeOH): 254, 353; (NaOMe): 263, 409; (AlCl<sub>3</sub>): 270, 302, 365, 401; (AlCl<sub>3</sub>/HCl): 269, 368, 403; (NaOAc): 255, 301, 358; (NaOAc/H<sub>3</sub>BO<sub>3</sub>): 254, 354. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>):  $\delta$  12.66 (1H, s, 5-OH), 8.02 (1H, br; H-2'), 7.57

(1H, d,  $J = 8.4$  Hz; H-6'), 6.90 (1H, d,  $J = 8.4$  Hz; H-5'), 6.74 (1H, br; H-8), 6.37 (1H, br; H-6), 5.47 (1H, d,  $J = 7.7$  Hz; H-1''), 4.41 (1H, br, H-1'''), 3.87 (3H, s, 7-OCH<sub>3</sub>), 3.86 (3H, s, 3'-OCH<sub>3</sub>), 1.04 (3H, d,  $J = 6$  Hz; H-6'''); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>):  $\delta$  177.9 (C-4), 165.5 (C-7), 161.3 (C-5), 157.2 (C-2), 156.8 (C-9), 150.8 (C-4'), 147.5 (C-3'), 133.7 (C-3), 122.5 (C-6'), 121.6 (C-1'), 115.6 (C-5'), 113.8 (C-2'), 105.4 (C-10), 101.3 (C-1''), 100.5 (C-1'''), 98.3 (C-6), 92.8 (C-8), 74.1 (C-3''), 73.3 (C-5''), 72.2 (C-4''), 71.5 (C-3'''), 71.0 (C-2''), 70.7 (C-2'''), 68.7 (C-4''), 68.4 (C-5'''), 65.7 (C-6''), 56.5 (7-OCH<sub>3</sub>), 56.3 (3'-OCH<sub>3</sub>), 18.3 (C-6'''). ESI-MS:  $m/z$  661 [M+Na]<sup>+</sup> (100%), 1299 [2M+Na]<sup>+</sup> (11%), 637 [M-H]<sup>-</sup> (100%).

*Isorhamnetin 3-O-(6''-O- $\alpha$ -rhamnosyl)- $\beta$ -galactoside (4)*

Yellow powder; UV  $\lambda_{\max}$  (MeOH): 254, 355; (NaOMe): 272, 326, 414; (AlCl<sub>3</sub>): 268, 302, 369, 400; (AlCl<sub>3</sub>/HCl): 268, 300, 363, 401; (NaOAc): 273, 323, 372; (NaOAc/H<sub>3</sub>BO<sub>3</sub>): 255, 357. <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>):  $\delta$  12.58 (1H, s, 5-OH), 8.00 (1H, br; H-2'), 7.51 (1H, d,  $J = 8.4$  Hz; H-6'), 6.91 (1H, d,  $J = 8.4$  Hz; H-5'), 6.41 (1H, br; H-8), 6.18 (1H, br; H-6), 5.46 (1H, d,  $J = 7.7$  Hz; H-1''), 4.43 (1H, br, H-1'''), 3.85 (3H, s, 3'-OCH<sub>3</sub>), 1.06 (3H, d,  $J = 6$  Hz; H-6'''); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>):  $\delta$  177.6 (C-4), 165.4 (C-7), 161.6 (C-5), 156.9 (C-2), 156.7 (C-9), 149.9 (C-4'), 147.4 (C-3'), 133.5 (C-3), 122.3 (C-6'), 121.4 (C-1'), 115.6 (C-5'), 113.8 (C-2'), 104.1 (C-10), 102.3 (C-1''), 100.5 (C-1'''), 99.3 (C-6), 94.3 (C-8), 74.0 (C-3''), 73.3 (C-5''), 72.3 (C-4''), 71.5 (C-3'''), 71.0 (C-2''), 70.8 (C-2'''), 68.7 (C-4''), 68.4 (C-5'''), 65.6 (C-6''), 56.1 (3'-OCH<sub>3</sub>), 18.3 (C-6'''). ESI-MS:  $m/z$  647 [M+Na]<sup>+</sup> (100%), 1271 [2M+Na]<sup>+</sup> (20%), 623 [M-H]<sup>-</sup> (100%) and ESI-MS/MS:  $m/z$  317 [M-sugar]<sup>+</sup>.

*Isorhamnetin 3-O-(2'',6''-O- $\alpha$ -di-rhamnosyl)- $\beta$ -galactoside (5)*

Pale yellow powder. TLC  $R_f$  0.41 (BAW). UV  $\lambda_{\max}$  (MeOH): 254, 354; (NaOMe): 272, 323, 401; (AlCl<sub>3</sub>): 270, 302, 371, 404; (AlCl<sub>3</sub>/HCl): 269, 361, 401; (NaOAc): 274, 321, 366; (NaOAc/H<sub>3</sub>BO<sub>3</sub>): 254, 353. <sup>1</sup>H- and <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>) see table (S1). ESI-MS:  $m/z$  793 [M+Na]<sup>+</sup>, 769 [M-H]<sup>-</sup>, and ESI-MS/MS:  $m/z$  317 [M-sugar]<sup>+</sup>.

## Supplementary material

**Figure S1:** Selected HMBC correlations of compound **1**

**Figure S2:**  $^1\text{H-NMR}$  spectrum of compound **1** in  $\text{DMSO-}d_6$

**Figure S3:**  $^{13}\text{C-NMR}$  spectrum of compound **1** in  $\text{DMSO-}d_6$

**Figure S4:** HH-COSY NMR spectrum of compound **1** in  $\text{DMSO-}d_6$

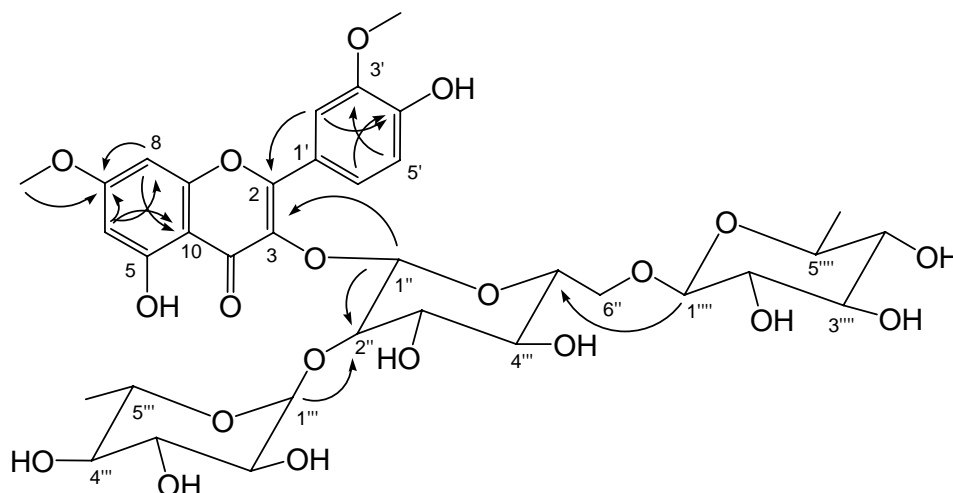
**Figure S5:** HSQC NMR spectrum of compound **1** in  $\text{DMSO-}d_6$

**Figure S6:** HMBC NMR spectrum of compound **1** in  $\text{DMSO-}d_6$

**Figure S7:** (+) ESI-MS spectrum of compound **1**

**Figure S8:** (-) ESI-MS spectrum of compound **1**

**Figure S9:** UV spectra of compound **1**



**Figure S1:** Selected HMBC correlations of compound **1**

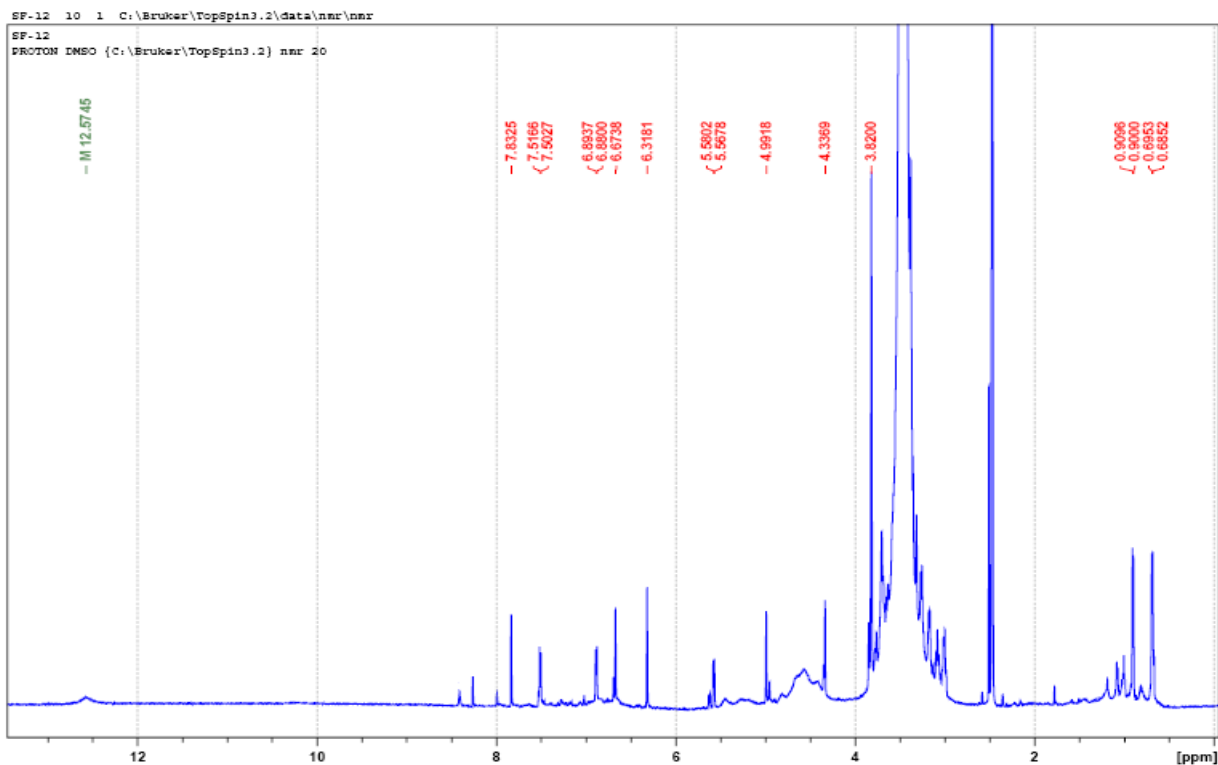


Figure S2:  $^1\text{H}$ -NMR spectrum of compound **1** in  $\text{DMSO-}d_6$

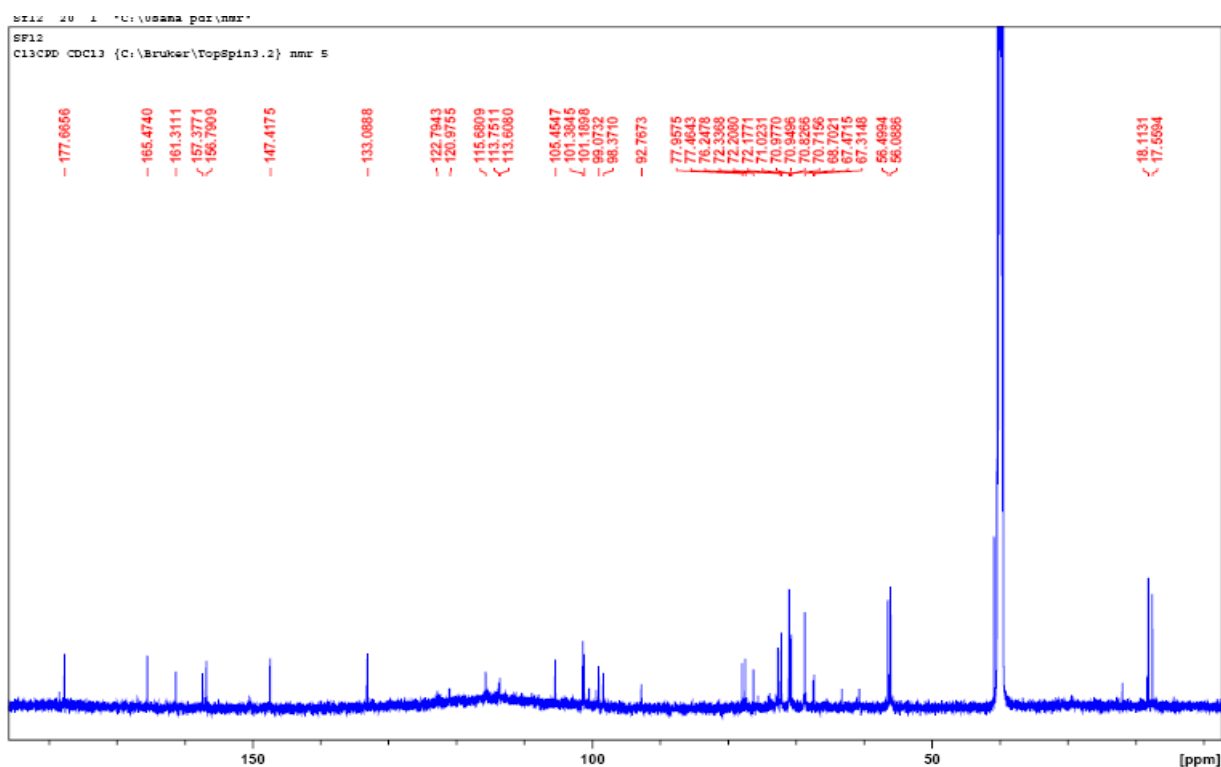


Figure S3:  $^{13}\text{C}$ -NMR spectrum of compound **1** in  $\text{DMSO-}d_6$

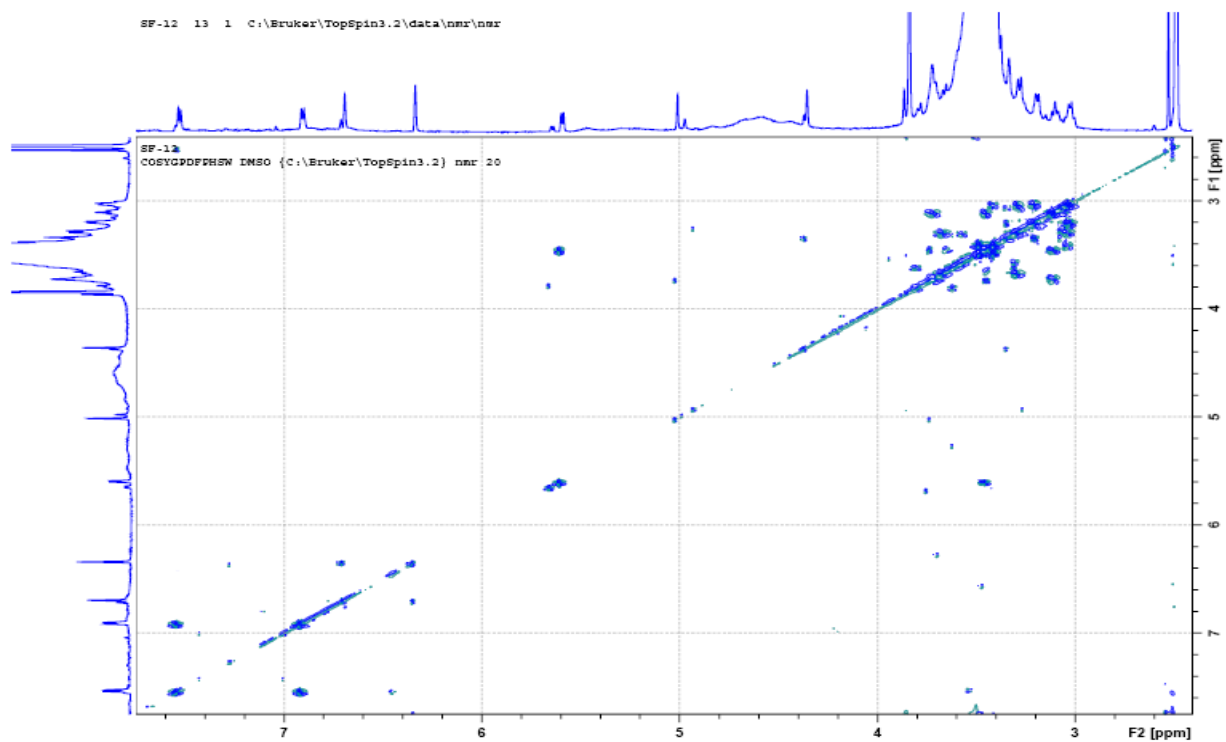


Figure S4: HH-COSY NMR spectrum of compound **1** in DMSO- $d_6$

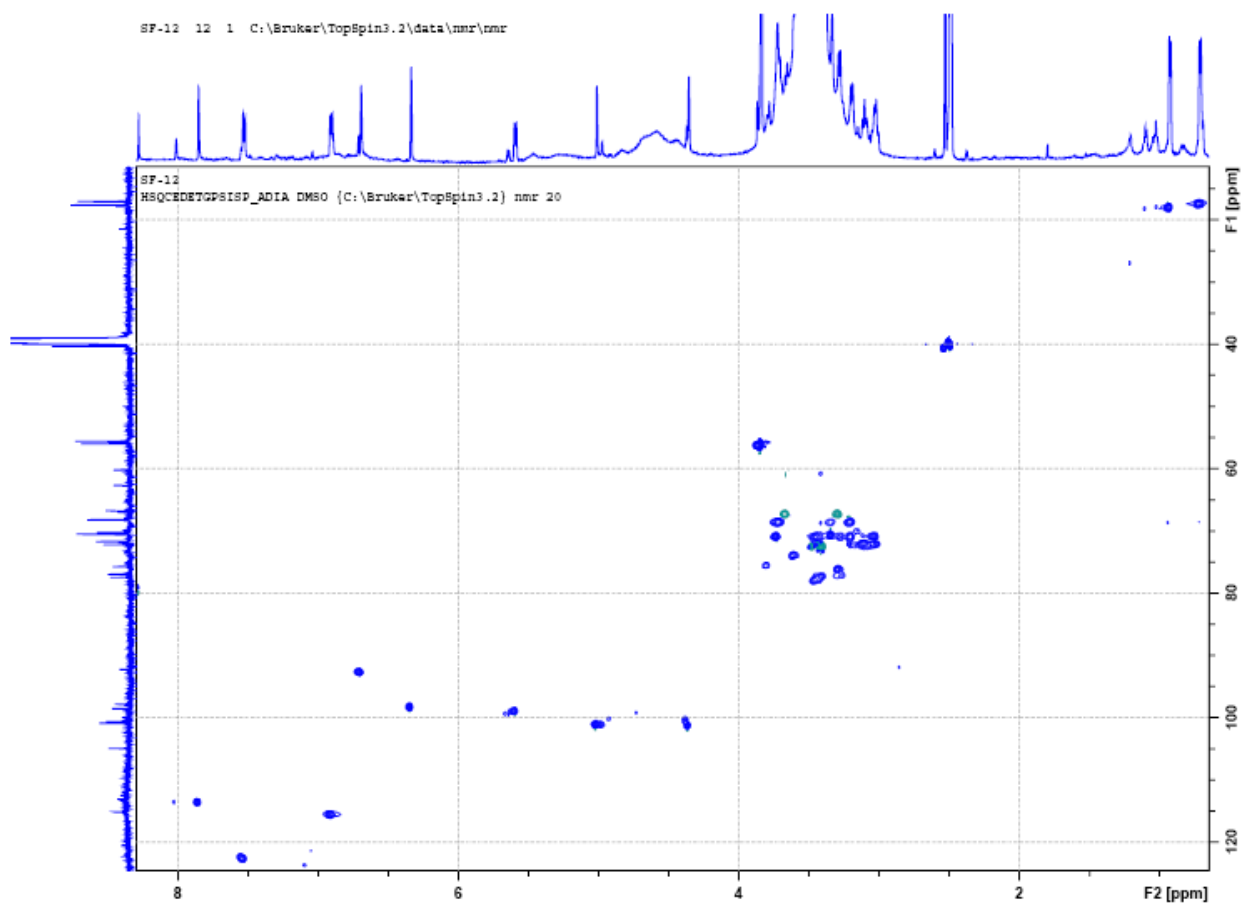
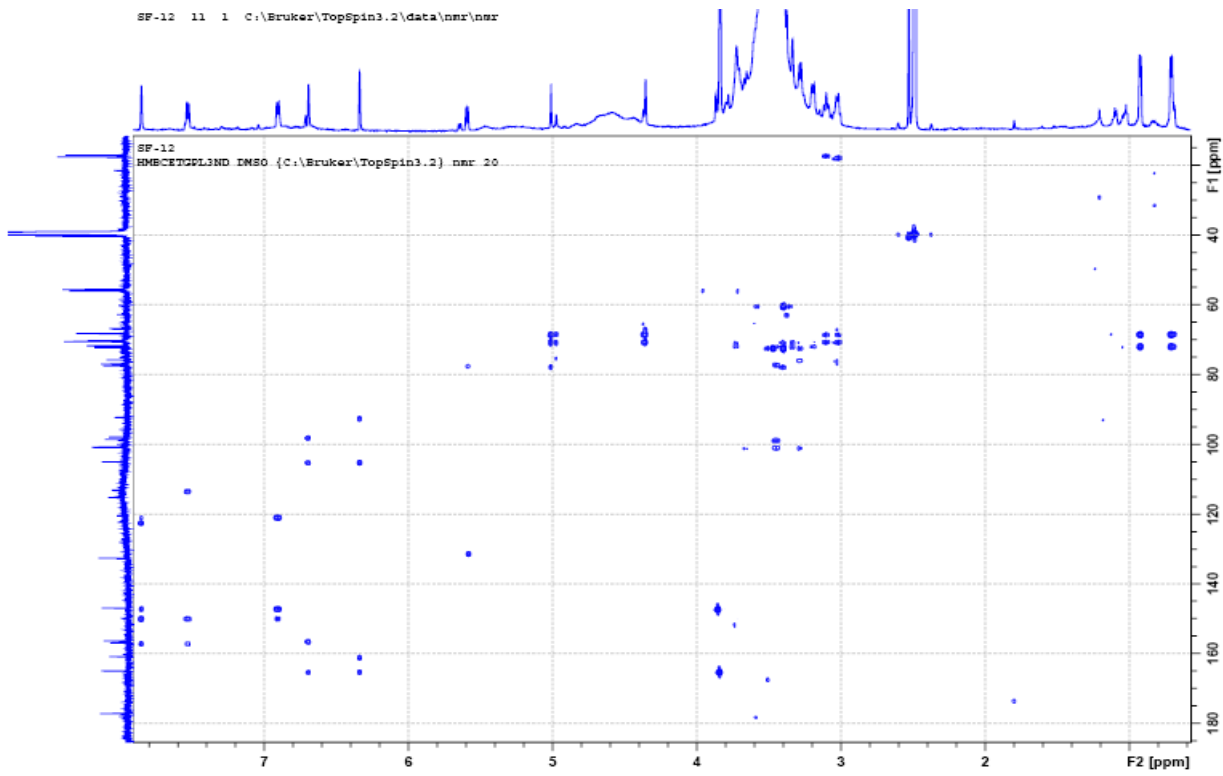
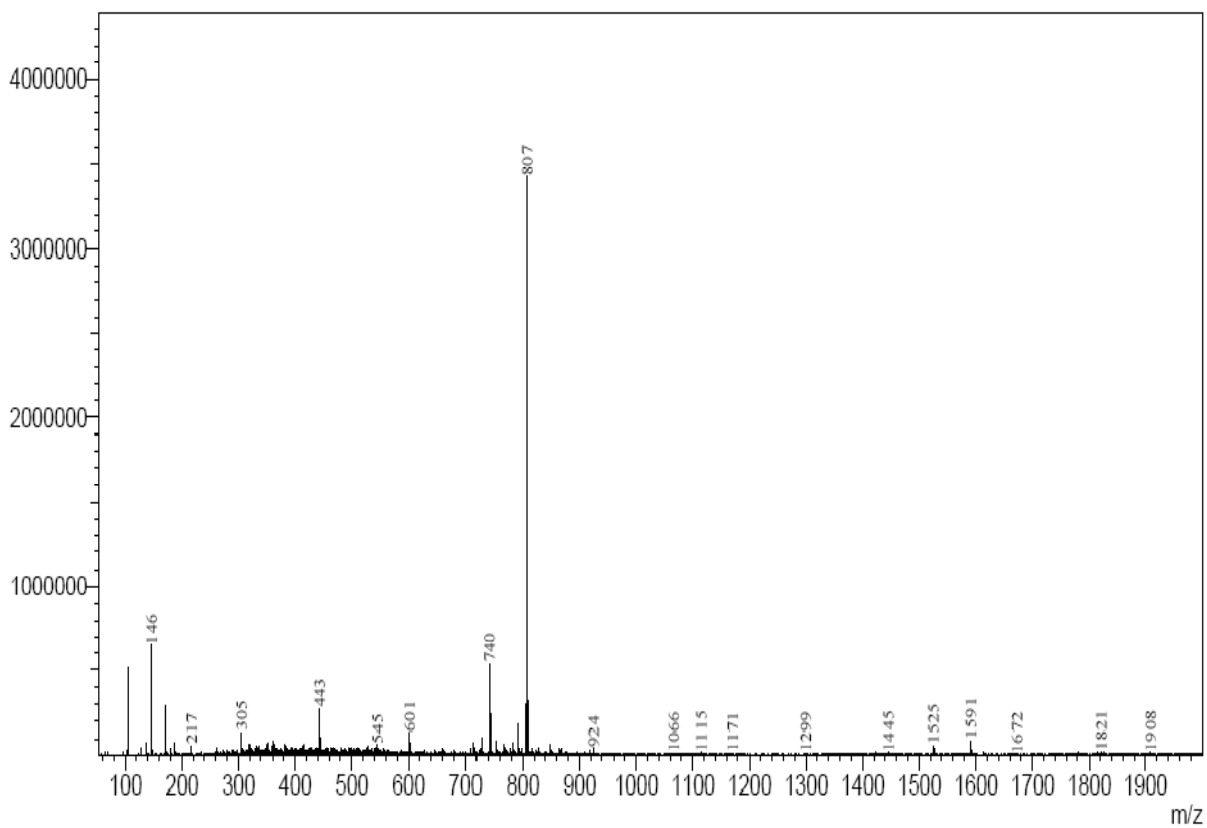


Figure S5: HSQC NMR spectrum of compound **1** in DMSO- $d_6$



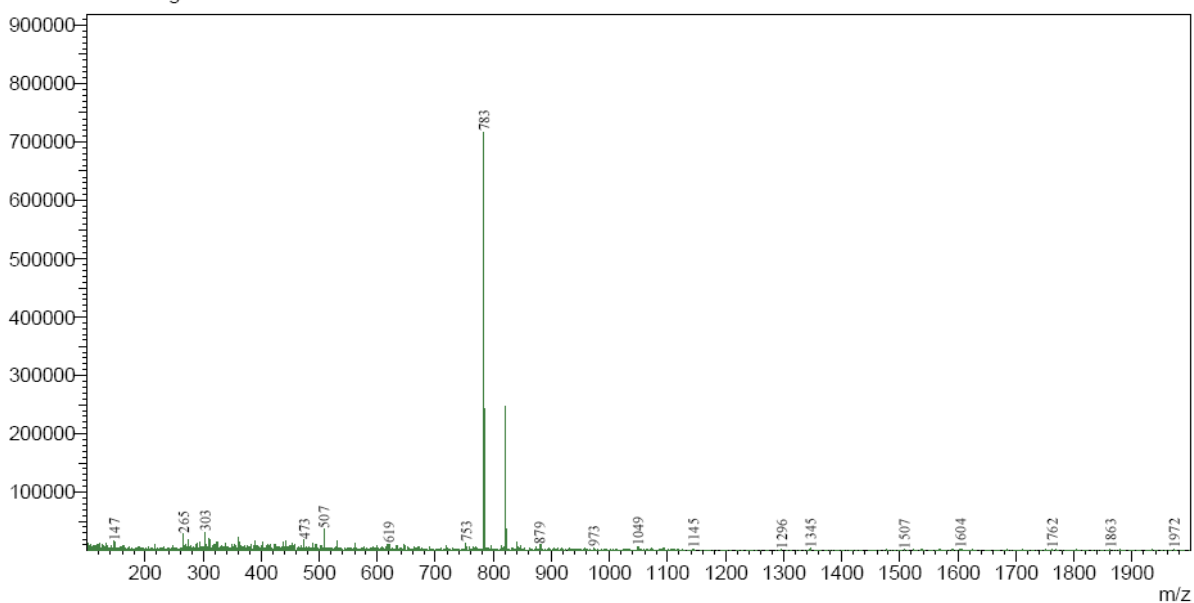
**Figure S6:** HMBC NMR spectrum of compound **1** in DMSO- $d_6$



**Figure S7:** (+) ESI-MS spectrum of compound **1**

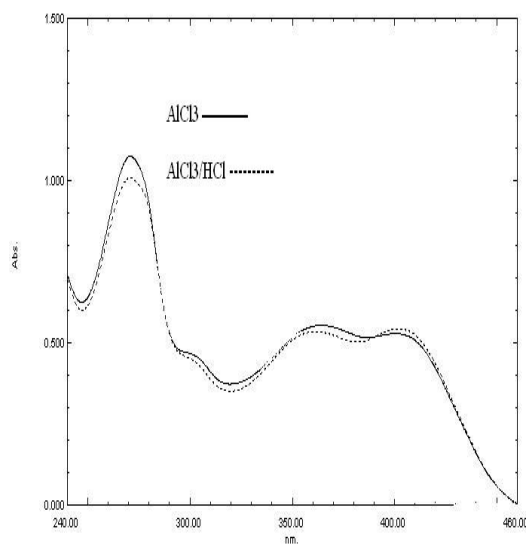
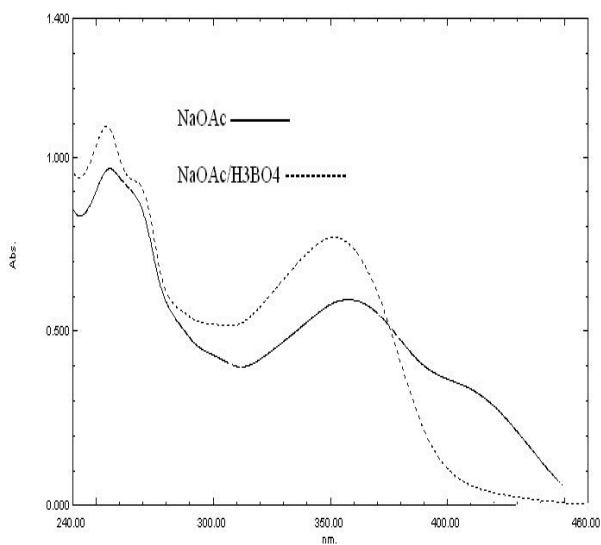
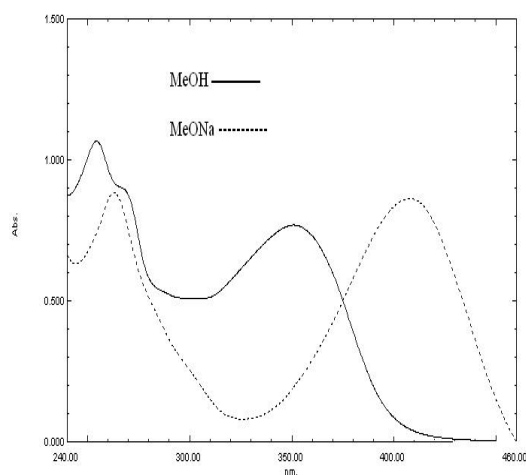


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**Figure S8:** (-) ESI-MS spectrum of compound **1**

**MeOH:** 254, 353  
**MeONa:** 263, 407  
**AlCl<sub>3</sub>:** 270, 365, 400  
**AlCl<sub>3</sub>/HCl:** 270, 362, 401  
**AcONa:** 256, 357  
**AcONa/H<sub>3</sub>BO<sub>3</sub>:** 254, 351



**Figure S9:** UV spectra of compound **1**